Vol. XIII. *August, 1907.* *No. 3.*

BIOLOGICAL BULLETIN

THE INFLUENCE OF THE AMOUNT OF INJURY UPON THE RATE AND AMOUNT OF REGENERATION IN MANCASELLUS MACROURUS (GARMAN).[1]

MAX M. ELLIS.

I. INTRODUCTION.

Until Dr. Zeleny announced the results of his experiments on the fiddler crab, *Gelasimus*, and the brittle star, *Ophioglypha*, it was generally thought that an increase in the amount of injury would decrease the ability to repair injury, that is, it would retard regeneration. In these two forms he found that an increase in the degree of injury produced a corresponding increase in the rate of regeneration. Later he established the same principle as true for the common crawfish,[2] *Cambarus propinquus*.

From the crawfish experiment he concludes that "in series with the greater degree of injury each chela regenerates more rapidly than the single removed chela of the series with the lesser degree of injury." These three experiments present the idea that an increase in the amount of injury accelerates rather than retards regeneration. However, it is probable, as is indicated by Dr. Zeleny's work on the brittle star, that there is a degree of injury, a limit, beyond which this is not the case. In the work just mentioned,[3] when all five of the arms were removed the regeneration was slower than when four were removed.

The present experiments were made with the object of contributing some quantitative data concerning the relation of the amount of injury to the rate and amount of regeneration. The

[1] Contribution from the Zoological Laboratory of Indiana University. No. 85.

[2] *Jour. Ex. Zoöl.*, Vol. II., No. 3, '05.

[3] *Jour. Ex. Zoöl.*, Vol. II., No. 1.

form *Mancasellus* was chosen because it is abundant in this region. It regenerates lost parts readily and as each leg has a breaking joint at the coxal-thoracic articulation it is possible to make all operations uniform. The results obtained are, in a measure, parallel with Dr. Zeleny's work.

II. Methods.

The isopods used in these experiments were taken in a stream near the Indiana University campus at Bloomington, from a part not over three hundred feet in length. In this distance it is probable that they had been subjected to the same general conditions previous to the experiments.

Set I. — For Set I. several hundred specimens were collected on October 3, 1906, from which twenty normal males measuring between 10–13 mm. were selected. These were divided into four series. In series A a "standard" injury was established by the removal of the right sixth walking leg. In each of the remaining three series the operation was the infliction of the "standard" injury plus an "added" injury. As "added" injury the right fifth walking leg was removed in series B, the right fifth, fourth and third in series C and all of the right thoracic appendages including the cheliped in series D. The operation was made in each series by pinching the tip of the appendage to be removed till the animal cast it off at the breaking joint. The above system was repeated daily on a fresh catch of Isopods for five days, that is, until October 8, 1906, when the completed set consisted of four series of twenty-five individuals each. Each specimen was kept in a twelve-ounce saltmouth bottle, which was inclined a few degrees from the horizontal by the mouth resting on an inch block. The water was changed every six days. As food, the partially decayed leaves of the common elm were supplied. An excess of leaves was always present. Fourteen days after the date of operation each individual was killed.

Set. II. — The second set, series E, F and G, was planned differently in order to obtain a uniform relation between the time of the last moult and the date of operation. On January 24, 1907, several hundred isopods were collected and two hundred and fifty normal males measuring between 13–16 mm. selected. These were placed in individual bottles at once. Twenty-four

hours later they were examined and twenty found to have moulted. These twenty were isolated for another twenty-four hours. At the end of the second day, that is, not more than forty-eight hours after their last moult, twelve individuals of about the same size from this twenty were operated upon. Thus a double check was made on the size of the specimens for only those whose length, both before and after the moult was about he same, were retained. Three series were used. In series E the right sixth walking leg was removed to again establish a "standard" series.

In the other two series "added" injury was also inflicted. The removal of right fifth and fourth walking legs constituted this "added" injury in series F and all right walking legs in series G. In the above manner twenty-one individuals were selected and operated upon on the twenty-sixth, nine on the twenty-seventh and eighteen on the twenty-eighth, giving a completed series of sixty individuals, twenty to a series. These series were maintained in the same manner as Set I., save that the water was changed daily. The method of operation was also the same. Two days after their next moult the isopods were killed. By February 11, 1907, twenty-eight had moulted and the set was discontinued.

III. Data.

The length in millimeters of the body, the original leg and the regenerated leg is given for each individual that had regenerated at the close of the experiment. The specific amount of regeneration, which is the per cent. of regeneration in terms of the original leg is given for both sets. The specific rate, which is the specific amount divided by the number of days in the moulting period was obtainable only for Set II.

Explanation of Tables.[1]

Set I.	Series A.	Right sixth walking leg removed.
	Series B.	Right 6–5 walking legs removed.
	Series C.	Right 6–5–4–3 walking legs removed.
	Series D.	Right 6–5–4–3–2–1 walking legs and cheliped removed.
Set II.	Series E.	Right sixth walking leg removed.
	Serses F.	Right 6–5–4 walking legs removed.
	Series G.	Right 6–5–4–3–2–1 walking legs removed.

[1] Abbreviations: Orig., original; Reg., regenerated; Spec. Amt., specific amount; Spec. Rate, specific rate; 13 +, moulted during last half of thirteenth day.

TABLE I.

SET I. *Series A.*

Cat. No.	Body Length.	Right Sixth Walking Leg.		Spec. Amt.	Moulting Period—Days.	
		Orig.	Reg		1st.	2d.
1	9	3.48	2.52	.72	13	
2	9	4.00	3.19	.80	2	
3	10	3.76	2.82	.76	13	
4	10	3.57	2.48	.69	12	
5	10	4.00	2.71	.67	13	
6	10	3.38	2.90	.86	13+	
7	10	3.76	2.52	.67	7	11
8	10	4.33	2.81	.65	13	
9	11	3.38	1.81	.54	12	
10	11	5.33	2.38	.45	13	
11	12	4.66	3.19	.69	13+	
Av.	10.1			.68		

Series B.

1	10	4.14	2.86	.69	13	
2	10	3.43	2.76	.80	11	
3	10	3.76	2.71	.72	9	
4	11	3.43	2.76	.80	1	8
5	11	4.29	2.19	.51	13+	
6	11	3.67	2.38	.68	8	
7	11	3.52	3.29	.93	4	
8	11	4.05	2.57	.63	13+	
9	11	3.19	2.57	.81	8	
10	11	4.38	2.76	.63	13	
11	12	3.67	3.43	.93	13	
Av.	10.8			.739		

TABLE II.

SET I. *Series C.*

Cat. No.	Body Length.	Right Sixth Walking Leg.		Spec. Amt.	Moulting Period—Days.	
		Orig.	Reg.		1st.	2d.
1	10	4.48	2.82	.63	13	
2	10	3.62	2.86	.79	7	13+
3	10	3.52	2.05	.58	11	
4	11	4.05	2.57	.63	2	13+
5	11	4.29	2.81	.65	13+	
6	11	4.14	2.86	.69	12	
7	12	4.00	3.10	.775	3	10
8	12	4.43	2.95	.86	9	
9	12	4.48	2.43	.54	10	
10	13	4.29	2.82	.88	2	13
Av.	11.2			.703		

Series D.

1	9	3.38	2.33	.66	10	
2	10	3.62	2.57	.71	13	
3	10	3.95	2.76	.69	3	
4	10	3.29	2.52	.77	12	
5	10	3.86	1.91	.49	7	
6	11	4.10	3.33	.81	8	
7	11	3.90	2.71	.72	7	13+
8	11	4.19	2.90	.69	12	
9	12	4.19	3.19	.76	12	
10	12	4.61	3.19	.67	11	
11	13	4.24	1.91	.45	7	
12	13	5.23	2.90	.55	13	
Av.	11.0			.664		

TABLE III.

SET II. *Series E.*

Cat. No.	Body Length.	Right Sixth Walking Leg.		Spec. Amt.	Moulting Period.	Spec. Rate.
		Orig.	Reg.			
1	15	7.25	2.90	.40	12	.033
2	14	5.86	2.38	.41	10	.041
3	14	5.76	2.71	.48	13	.037
4	13	6.00	2.57	.43	14	.031
5	13	5.76	2.71	.48	13	.037
Av.	13.8			.441	12.4	.036

Series F.

1	16	7.19	3.10	.43	17	.025
2	15	7.00	2.95	.42	16	.026
3	15	7.00	3.14	.45	15	.030
4	14	5.67	3.48	.61	14	.044
5	14	6.95	3.00	.43	16	.027
6	14	6.48	3.00	.46	16	.029
7	13	6.14	3.10	.50	17	.029
8	13	5.38	2.43	.45	13	.035
9	13	5.57	2.14	.38	16	.024
Av.	14			.48	15.5	.030

Series G.

1	16	6.48	3.10	.48	15	.032
2	15	7.00	2.57	.37	12	.031
3	15	6.67	2.71	.41	13	.031
4	15	6.24	2.95	.47	13	.036
5	14	6.90	3.14	.46	17	.027
6	14	6.00	2.62	.44	15	.029
7	14	5.33	2.33	.44	16	.028
8	14	5.86	2.67	.46	14	.033
9	14	5.05	2.76	.55	13	.042
10	14	4.95	2.90	.59	15	.045
11	14	6.48	3.10	.45	13	.035
12	14	6.67	2.90	.44	16	.028
13	14	6.43	2.95	.46	13	.044
14	14	4.76	2.29	.48	10	.048
Av.	14.3			.463	13.5	.035

IV. Results.

1. *Specific Amount of Regeneration.*

Set. I. A — .67, B — .74, C — .70, D — .66.

Starting with the standard series A the specific amount of regeneration increases in series B and decreases in series C and D. Both B and C are greater than A, while D is less than A. These values show two important facts: (1) that there is an optimum degree of injury and (2) that there is a limit to the amount of added injury that may be inflicted and the resultant regeneration still be greater than that following the standard injury. This limit of added injury is between C and D.

Set. II. E — .44, F — .48, G — .46.

The specific amounts of Set II. follow precisely the same rule of arrangement as those of Set. I. There is a rise and a fall in the amount of regeneration. However no series of Set. II. is below standard and as a result the limit of added injury does not occur. Since the injury inflicted in series A and E was the same, there being so few individuals in series E it was thought advisable to obtain the values of Set II. in terms of Set I. Accordingly a coefficient (1.542) was established by dividing the specific amount of series A by that of series E. The specific amounts of Set II. were then multiplied by this and the following table made by placing the values of both sets in the order of amount of injury.

A and E — .68, B — .74, F — .74, C — .70, G — .70, D — .66.

In this table of the combined values there is the same plan of increase and decrease in the amount of regeneration as has been noted in both sets. It is a rise at the first followed by a steady decline. The limit of added injury and the optimum are both present.

Considering these tables three things are evident:

1. The amount of regeneration increases directly with the amount of injury until an optimum has been attained.

2. Beyond the optimum added injury still gives an amount of regeneration greater than that of the standard injury up to a limit.

3. Beyond this limit regeneration is less than the standard.

2. *Specific Rate.*

The specific rates for Set I. were not obtainable as the time and not the moult was constant. For Set II. the specific rates were E — .036, F — .030, G — .035. Nothing very certain can be said as to the value of these figures, however, as specific rate may be an unreliable quantity. The greatest source of error in computing it is the moulting period. This could easily be influenced either by (1) the shock of the operation, or (2) by the asymmetrical condition produced by the loss of appendages. The effect of neither was determined, yet because of their existence as possible factors in the rate of moulting the exact worth of the specific rate is not known.

V. Conclusion.

From the data collected it seems probable that an increase in the amount of injury produces an increase in the amount of regeneration until a certain limit is reached. This limit of added injury is probably constant for the species.

As a point of interest it may be noted that the individuals collected in October regenerated about fifty per cent. more in a given time than those collected in January. It seems that the season of the year may have some influence upon regeneration.

VI. Summary.

1. Each leg of *Mancasellus* possesses a breaking joint at the coxal-thoracic articulation.

2. The season of the year may influence the ability to regenerate lost parts.

3. Increased injury increases the amount of regeneration in *Mancasellus* until the optimum is reached. From this it decreases to a limit beyond which the amount of regeneration is less than that of the standard.

4. The optimum seems to be low for *Mancasellus*.

5. The limit of added injury is relatively high.

Acknowledgment.

This work was undertaken and carried out at the suggestion of Dr. Charles Zeleny. I thank him for his willing assistance.